

REMARKS

Interview

Applicants would like to express their appreciation for the courtesy extended by Examiner Kruse and Examiner Nelson to Angela Dallas Sebor, Donald Weeks, Dipanjan Nag and Richard Shear during the personal interview of January 5, 2004. During the interview, the outstanding issues under 35 U.S.C. § 112, first paragraph were discussed. This supplemental amendment and response represents the amendments discussed with and proposed by the Examiners during the interview and also presents the additional publications directed to vanillate oxygenase that were requested by the Examiners.

Claim Amendments

The claims have been amended to clarify the claims as discussed in the January 5 interview. Initially, to expedite prosecution, Applicants have cancelled non-sequence-based Claims 1, 4, 59, 63, 66 and 67 without prejudice to or disclaimer of the subject matter therein. Other claims that previously depended from these cancelled claims have been amended to depend from the appropriate sequence-based claim. Second, as discussed with the Examiners, to expedite prosecution, reference to fragments of the claimed sequences has been removed from the claims. It is noted that none of the above-identified amendments are to be construed as an admission by Applicants regarding the patentability of the claims. Applicants expressly reserve the right to pursue all cancelled subject matter in a continuation application.

The claims have also been amended as discussed with the Examiner to more particularly point out the features of the oxygenase that correlate the structure with the function of the oxygenase. In particular, the claims were amended in the Amendment submitted November 29, 2004 to recite that the claimed oxygenase comprises an iron-sulfur cluster. In this supplemental Amendment, the claims have been further amended to recite that the claimed oxygenase binds free Fe^{2+} (i.e., the oxygenase is taught to require added Fe^{2+} for activity), thus denoting the iron binding region. Support for this additional amendment is found in the specification on page 52, lines 15-18.

Rejections Under 35 U.S.C. § 112, First Paragraph:

Initially, and to avoid repetition of all of the detailed arguments here, the Examiner is respectfully first referred to the arguments made with respect to the written description and enablement rejections in the November 29, 2004 Amendment and Response, and to the publications that were attached in support of these arguments. It is believed that the Examiner has copies of all of the publications discussed below, most of which were submitted in the November 29 response, with the exception of the Brunel and Davison publication which is enclosed with this response, and the Jiang et al. publication which was submitted in the December 13, 2002 response.

As discussed with the Examiners in the January 5 interview, and as set forth in the prior responses, particularly the November 29 response, at the time of the present invention, there was a *substantial* amount of art-recognized correlation and relationship between the structure of Rieske non-heme iron-binding oxygenases that transfer electrons through iron-sulfur clusters and free iron atoms and the function of such enzymes. As previously stated, the oxygenase of the present invention falls within this class of oxygenases (see specification, page 40, line 27 to page 41, line 1; page 46, lines 12-23; page 47, line 27 to page 48, line 18; page 49, lines 20-26; page 52, lines 8-10; page 54, lines 3-6). In particular, examination of the biochemical and physical properties of oxygenase_{DIC}, as well as its primary amino acid sequence, revealed that it contains two structural elements outside of the substrate binding region that are essential to the function of the enzyme: (1) a Rieske iron-sulfur cluster (see the specification at page 40, line 27 to page 41, line 1, wherein this iron-sulfur cluster is first identified), and (2) a requirement for iron (Fe²⁺) for activity (page 52, lines 15-18, for example), which the art recognizes in the structure of the oxygenase as a mononuclear iron binding site (see, *e.g.*, page 292, last three paragraphs, and page 294 of Mason J.R. and Cammack R. (1992) *Annu Rev Microbiol.* 46:277-305; and see also Fig. 3 of Jiang et al., 1996, *J. Bacteriol.* 178:3133-3139).

Finally, it was known in the art at the time of the invention that the binding site for free iron is situated in immediate proximity to the portion of the substrate that is modified in the chemical reaction catalyzed by the oxygenase_{DIC} (i.e., the O-methyl group of dicamba) (see, *e.g.*, page 294, Mason J.R. and Cammack R. (1992), *supra*). The art at the time of the invention recognized the consensus binding sites for the Rieske iron-sulfur clusters, and binding sites for the free iron in Rieske non-heme iron oxygenases were widely known (See, *e.g.*, Mason J.R. and Cammack R.

(1992), *supra*; Butler, C. S. and Mason, J. R. (1997) *Advances in Microbial Physiology* 38, 47-84; Neidle et al. (1991) *J. Bacteriol.* 173(17):5385-5395; Junker et al. (1997) *J. Bacteriol.* 179(3):919-927; Gurbiel et al. (1996) *Biochemistry* 35:7834-7845; Harayama et al. (1992) *Annu Rev Microbiol.* 46:565-601; Jiang et al., 1996, *supra*).

Referring again to these specific structural regions, which have been illustrated and discussed in the Weeks December 2002 Declaration in comparison to other oxygenases of the same or similar class that were known at the time of the invention (see Figure A), account for the majority of the functional capability of the claimed oxygenase and the identifying motifs for such structures were known in the art at the time of the invention.

Applicants also reference the prior submitted publication of Jiang et al. (1996), *supra* because it provides an additional illustration that the structures for the iron-sulfur cluster and mononuclear non-heme iron binding site were recognized in the art at the time of the invention for a variety of oxygenases, and particularly includes the vanillate demethylase (VanA), which is the oxygenase to which the claimed dicamba-degrading oxygenase is most homologous (at 33.8% identity).

As discussed and suggested in part by the Examiners during the January 5 interview, the presently claimed oxygenase is clearly described and identified both structurally and functionally as a member of the genus of dicamba-degrading oxygenases having 65% identity to SEQ ID NO:4 according to the following points:

- **primary sequence** - The claimed oxygenase has 65% identity to SEQ ID NO:4, whereas the next closest structural homologue of SEQ ID NO:4 known in the art is only 33.8% identical (i.e., the oxygenase from vanillate demethylase; see specification page 54, lines 3-6). This has been substantiated by the Examiner's own sequence searches, as discussed in the January 5 interview. Therefore, much of the sequence that is presently claimed, including sequences having 65% identity to SEQ ID NO:4, is unique to the class of dicamba-degrading oxygenases, even *without* the additional structural and functional characteristics that are presently recited in the claims.
- **iron-sulfur cluster** - The claimed oxygenase contains an iron-sulfur cluster, which is required for the electron transfer that is essential to the function of the enzyme, and this was noted in the specification as discussed above. Since the art at the time of the invention recognized the "signature motif" of such a cluster (e.g., see Fig. 3, Fig. 4 and pages 292-294

of Mason J.R. and Cammack R. (1992), *supra*; or pages 61-71 of Butler, C. S. and Mason, J. R. (1997), *supra*; page 5389, last paragraph, and Fig. 3 and Fig. 4 of Neidle et al. (1991), *supra*; or Fig. 3 of Jiang et al. (1996), *supra*), one of skill in the art can readily identify this structure in SEQ ID NO:4 (and also therefore avoid modifications to this site that would be predicted to disrupt functionality of the enzyme).

- **free iron (Fe^{2+}) binding** - The claimed oxygenase has a requirement to bind free iron (i.e., Fe^{2+} , noted in the specification as discussed above), and therefore has a free iron binding site, as do the other oxygenases in the class of oxygenases that degrade aromatic compounds. Again, the art at the time of the invention recognized the "signature motif" of such a binding site (see, e.g., page 294 of Mason J.R. and Cammack R. (1992), *supra*; or page 72-75 of Butler, C. S. and Mason, J. R. (1997), *supra*; page 5390, last full paragraph and Fig. 3 of Neidle et al. (1991), *supra*; or Fig. 3 of Jiang et al. (1996), *supra*). Therefore, one of skill in the art can readily identify this structure and avoid modifications to this site that would be predicted to disrupt functionality of the enzyme).

- **substrate**

- The claimed oxygenase binds to dicamba, and the art recognizes that the substrate of the Rieske non-heme oxygenases must bind near the free iron, because the oxidation of the free iron activates the oxygen, which results in hydroxylation of the substrate (i.e., the components must be situated in close proximity for the reaction to occur) (see, e.g., page 294, Mason J.R. and Cammack R. (1992), *supra*; or page 73, first full paragraph of Butler, C. S. and Mason, J. R. (1997), *supra*). Therefore, one of skill in the art can readily identify what portions of the oxygenase structure lie in proximity to the free iron binding site and also avoid modifications to the region of the structure that would be predicted to disrupt functionality.


- The claimed oxygenase binds a substrate (dicamba) that is in the class of benzoic acid herbicides. The class of oxygenases of which the Rieske oxygenases, and specifically the oxygenase_{DIC}, are members are known in the art to degrade compounds containing a benzene nucleus - thus the substrate structures are similar (see again Mason J.R. and Cammack R. (1992), *supra*). Indeed, the substrates for the most homologous oxygenase to oxygenase_{DIC} (i.e., vanillate oxygenase), are vanillate (see attached publication by Brunel and

Davison (1988, *J. Bacteriol.*, 170:4924-4930) and anisic acid. These substrates are highly similar in structure to dicamba (see attached document which shows the side-by-side comparison of these known substrate structures). This illustrates the point that the amino acids in the substrate binding region that account for the dicamba-binding specificity will be expected to be very few, since structures bound by different oxygenases are quite similar. Furthermore, among oxygenases, it was known in the art at the time of the invention that the range of substrates is often quite large, which is an indication that the substrate binding region lacks significant geometric specificity (See *e.g.*, ., Mason J.R, and Cammack R. (1992) *Annu Rev Microbiol.* 46:277-305; or Butler, C. S. and Mason, J. R. (1997) *Advances in Microbial Physiology* 38, 47-84). Therefore, one of skill in the art recognizes that the portion of the oxygenase that is specific for binding to dicamba is not extensive because *there are only a few amino acids that are expected to form direct contacts with the specific dicamba substrate.* Other surrounding amino acids often can be changed without significantly affecting the ability of the enzyme to bind the substrate and to catalyze the usual reaction involving the substrate.

In summary, Applicants submit that the present specification and the knowledge in the art at the time of the invention allow one of skill in the art to envision the full scope of the oxygenase sequences as claimed, and then readily make and use such enzymes. In view of the foregoing remarks, in conjunction with those submitted in the November 29 Amendment and Response, Applicants respectfully request the withdrawal of all remaining rejections and expeditious allowance of the currently pending claims. In the event that the Examiner has any questions or concerns regarding this matter, he is encouraged to contact the below-named agent at (303) 863-9700 to expedite the allowance of the claims.

Respectfully submitted,

SHERIDAN ROSS P.C.

By: 
Angela Dallas Sebor
Registration No. 42,460
1560 Broadway, Suite 1200
Denver, CO 80202-5141
(303) 863-9700

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